Sleep Medicine Reviews 20 (2015) 27–45

Contents lists available at ScienceDirect

Sleep Medicine Reviews

journal homepage: www.elsevier.com/locate/smrv

CLINICAL REVIEW

Oxidative stress in obstructive sleep apnea and intermittent hypoxia – Revisited – The bad ugly and good: Implications to the heart and brain

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ARTICLE INFO

Article history:
Received 3 March 2014
Received in revised form
13 July 2014
Accepted 14 July 2014
Available online 24 July 2014

Keywords:
Obstructive sleep apnea
Oxidative stress
ROS/RNS
Anti-oxidants
Ischemia/reperfusion
Ischemic preconditioning
Acute myocardial infarction
Stroke
Endothelial progenitor cells
Coronary collaterals

SUMMARY

Obstructive sleep apnea (OSA), characterized by intermittent hypoxia (IH), is linked with increased reactive oxygen species/reactive nitrogen species (ROS/RNS) and oxidative stress, which adversely affect the associated cardio-/cerebro-vascular disease in OSA. Yet, animal and a small number of human studies support activation of cardio-/cerebro-protective mechanisms as well. ROS/RNS are intricate and multifaceted molecules with multiple functions. At low-moderate concentrations ROS/RNS are considered "good", by regulating vital cellular functions. At higher levels, they are considered "bad" by promoting oxidative stress and damaging vital macromolecules through ischemia and reperfusion (I/R) injury. Subsequently, ROS/RNS can get "ugly" by eliciting sterile inflammation and a multitude of deadly pathologies. What makes ROS/RNS good, bad, or ugly? A dynamic interplay between a large number of factors determines the outcomes. These include the types of ROS/RNS produced, their quantity, duration, frequency, intracellular localization, micro-environmental antioxidants, as well as the genetic make-up and life style related variables. This review presents the currently available data on redox biology in physiological/pathophysiological conditions and in OSA/IH, in order to better understand the apparently contradictory findings on damage vs. repair. These findings are discussed within the context of the prevailing views on I/R associated ROS/RNS, and their potential implications to OSA.

Introduction

Obstructive sleep apnea (OSA) is a highly prevalent breathing disorder in sleep. It is characterized by intermittent hypoxia (IH) leading to blood hypoxemia, hypercapnia, sleep fragmentation, augmented respiratory efforts and increased sympathetic activity [1]. At least 4% and 2% of adult men and women of the general population are diagnosed with OSA and its characteristic symptoms [1]. The prevalence of sleep disordered breathing (SDB) in men and women not displaying day time somnolence may rise up to 24% and 10%, respectively. In obese and elderly populations these values rise to 60% [2]. OSA is also an independent risk factor for cardiovascular morbidity [3–5], and its prevalence is higher than 60% in patients after acute myocardial infarction (AMI) or stroke [6,7]. Moreover, the incidence of cardiovascular morbidities such as hypertension, ischemic heart disease, chronic heart failure, arrhythmias and strokes was also shown to be higher than in the general population [3], thus, making OSA a major public health problem by affecting patient's health and quality of life [8]. These latter findings prompted a great number of studies over the past decade aimed at elucidating the impact of OSA on the cardio- and cerebro-vascular system and the associated comorbidities. However, the underlying mechanisms of this association are complex and intertwined and not entirely understood.

Oxidative stress and concomitant inflammation are two of the prominent underlying mechanisms suggested to explain this association. The former is defined as an imbalance between pro-oxidant and anti-oxidant systems resulting in excessive production of reactive oxygen species (ROS). The latter is the body's response to a variety of external as well as internal insults including

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http://dx.doi.org/10.1016/j.smrv.2014.07.003
1087-0792/

Abbreviations

AHI	apnea–hypopnea index
AMI	acute myocardial infarction
AP1	activator protein1
BH_4	tetrahydrobiopterin
CAD	coronary artery disease
CD	cluster of differentiation
cGMP	cyclic GMP
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
eNOS	endothelial NOS (NOS3)
EPCs	endothelial progenitor cells
EPO	erythropoietin
Erk1/2	extracellular signal-regulated kinase
GPx	glutathione peroxidase
GSH	glutathione (reduced)
GSSG	glutathione disulfide (oxidized)
H_2O_2	hydrogen peroxide
HDL	high-density lipoprotein
HIF-1α	hypoxia inducible factor-1α
HNA	4-hydroxy-2-nonenal
HO-1	heme oxygenase 1
HSPs	heat shock proteins
ICAM-1	intracellular cell adhesion molecule 1
I/R	ischemia and reperfusion
IH	intermittent hypoxia
IL-8	interleukin 8
IL-6	interleukin 6
iNOS	inducible NOS (NOS2)
IPC	ischemic preconditioning
Keap1	Kelch-like ECH-associated protein 1
LAD	left anterior descending artery
LDL	low-density lipoprotein
MDA	malonaldehyde
MI	myocardial infarction
mPTP	mitochondrial permeability transition pore
NAC	N-acetylcysteine
NADPH	reduced nicotinamide adenine dinucleotide phosphate
nCPAP	nasal continuous positive airway pressure
NFκB	nuclear factor κB
nNOS	neuronal NOS (NOS1)
NO	nitric oxide
NOS	nitric oxide synthase
Nox	NADPH oxidase
Nrf2	nuclear factor (erythroid-derived 2)-like2
$O_2^{\bullet-}$	superoxide anion
ODI	oxygen desaturation index
$OH^{\bullet}$	hydroxyl radical
$OONO^-$	peroxynitrite
OSA	obstructive sleep apnea
oxLDL	oxidized LDL
p38 MAPK	p38 MAP kinase
PAC-1	specific marker for glycoprotein (GP)IIb/IIIa
PI3K	phosphatidylinositol-3-kinase
PKC	protein kinase C
PON-1	paraxonase-1
PSLG-1	P-selectin glycoprotein ligand 1
Redox	oxidation/reduction balance
RNS	reactive nitrogen species
ROS	reactive oxygen species
SDB	sleep disordered breathing
SH	thiol
SOD	superoxide dismutase
TBARs	thiobarbituric acid reactive substances
TNF-α	tumor necrosis factor α
VCAM-1	vascular cell adhesion molecule 1
VEGF	vascular endothelial growth factor
VEGF-R2 (KDR)	VEGF receptor 2

oxidative stress. This association between oxidative stress and inflammation makes both mechanisms tightly interconnected and exacerbating each other [9,10].

The involvement of oxidative stress and inflammation and their potential role in promoting cardiovascular morbidity in OSA were extensively described in a review published in this journal in 2003 [11]. In that paper it was suggested that intermittent hypoxia (IH) – the hallmark of OSA – characterized by profound hypoxic episodes followed intermittently by rapid blood oxygenations could be considered analogous to repeated ischemia and reperfusion (I/R) events which result in injury due to flux of ROS during the reperfusion period. I/R injury is a well-established oxidative stress pathway for generating endogenous ROS. It occurs when blood flow to tissues or organs is disrupted and subsequently restored [12]. In a similar manner, the nightly IH cycles OSA patients experience promote ROS production and oxidative stress through these pathways, as shown over the last decade [11,13].

ROS molecules damage a multitude of vital biomolecules, hence, affecting a vast number of pathologies. Therefore, they are considered "bad/ugly" [14]. Despite their injurious nature, by acting like a double-edged sword, ROS are also considered "good". While at **high quantities** ROS promote inflammation and injury, at **low or moderate concentrations**, ROS act in vital signaling pathways essential for repair and survival. This dual activity is exemplified in various morbidities. In cancer cells, for instance, ROS activate intracellular signaling cascades that maintain the oncogenic phenotype but also possess anti-tumorigenic activity by inducing cell death [15]. Ischemia and reperfusion (I/R) is another well-established phenomenon demonstrating the dual functions of ROS. Although I/R is mostly known as a pathway for eliciting ROS production and subsequent injury, paradoxically, in many instances several brief and intermittent cycles of I/R were shown to exert **protective** rather than damaging effects. These protective effects, termed **ischemic preconditioning (IPC)**, were demonstrated in various organs including the heart and brain.

Favorable/unfavorable effects of ROS in physiology and pathophysiology are two sides of the same coin [13]. Over the last decade, a great number of studies and reviews were dedicated to the unfavorable effects of IH-associated ROS, as oxidative stress, inflammation and the resultant cardio–cerebro-vascular morbidities in OSA. However, evidence supporting potential protective effects in OSA/SDB/IH is also emerging. It is mainly provided by controlled animal studies mimicking OSA and from recent cellular and epidemiological studies.

This review is aimed at integrating the currently acquired knowledge on redox biology with the currently emerging knowledge on redox biology in OSA/IH while focusing on the complexity of I/R associated damage and repair. This may allow to implement the massively acquired data demonstrating the intertwined unfavorable/favorable effects of redox biology and further stimulate the interest and understanding in this expanding field of research. As such, this may also facilitate the search for detecting potential markers for protective mechanisms associated with IH and OSA/SDB to identify who runs a greater or lower risk for associated morbidities.

Overview

Reactive oxygen species and oxidative stress have long been implicated in initiating and propagating inflammatory responses mediated by leukocyte activation and altered adaptive and immune/inflammatory signaling pathways. Thus, a great number of transcription factors and signaling pathways are modulated by ROS, the most prominent and relevant to OSA being hypoxia inducible factor-1α (HIF-1α), nuclear factor κB (NFκB), activator protein1 (AP1), and nuclear factor (erythroid-derived 2)-like2 (Nrf2) [16]. Thereby, in response to IH, leukocytes, platelets, and endothelial cells undergo activation and display an activated and a pro-inflammatory phenotype which facilitates increased production of inflammatory cytokines, adhesion molecules and additional ROS. Altogether, excessive ROS, increased expression of adhesion molecules, and inflammatory cytokines, diminish nitric oxide (NO) activity and promote endothelial dysfunction, which is the prelude to atherosclerosis. This sequence further promotes oxidative stress. The diminished NO levels which exacerbate adhesion molecule expression in leukocytes, platelets, and endothelial cells, promote leukocytes–endothelial cell interactions further enhancing endothelial dysfunction. Jointly, these processes lead to the development of atherosclerosis and eventually to cardiovascular morbidity. The sequence of events described, is illustrated in Fig. 1, based on Lavie [11]. To concur with the more recently acquired data, it highlights both inflammatory and protective pathways. Fig. 2 elaborates the inflammatory pathway through the involvement of the specific immune/inflammatory cells and molecules which contribute to a pro-inflammatory/pro-thrombotic phenotype in the vasculature in response to OSA/IH. This activated phenotype of the various circulating blood cells including monocytes, neutrophils, lymphocytes, dendritic cells and platelets promotes interactions and adhesion with endothelial cells. A great number of molecules are expressed by these activated cells and facilitate the interactions with activated endothelial cells. The most prominent being adhesion molecules such as selectins and integrins, ROS, NO, and inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8). Together, these complex interactions which damage the endothelium promote endothelial dysfunction and exacerbate atherosclerosis in OSA as well as in rodents treated with IH [17–19].

It should be noted that just over a decade ago "the potential role of ROS in OSA was mainly speculated based on theoretical considerations linking increased levels of free radicals in hypoxic conditions on one hand, and their proven involvement in inflammatory and atherosclerotic processes on the other" [11]. Yet, the supporting evidence in OSA was scarce [20–22]. To date, a PubMed search using the terms "oxidative stress and sleep apnea" or "inflammation and sleep apnea" shows an exponential growth since 2003. Hence, the last decade has witnessed an unprecedented number of

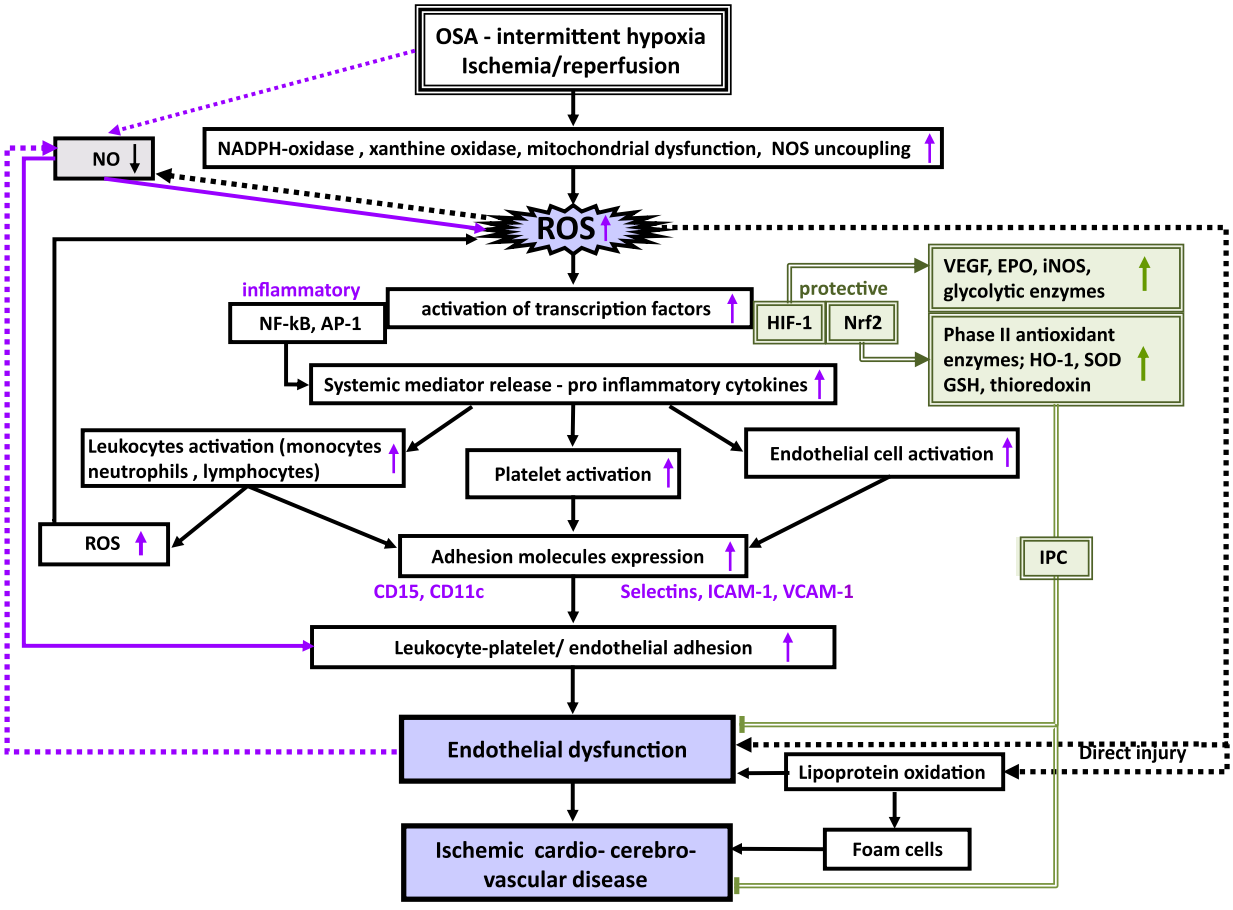

Fig. 1. The sequence of events in OSA starting from the nightly intermittent hypoxia and ending with endothelial dysfunction and vascular disease. In parallel activation of protective mechanisms as ischemic preconditioning to counteract ischemic cardio–cerebro-vascular disease. Green lines indicate protective pathways, (Modified from Lavie, [11]). AP1, activator protein1; CD, cluster of differentiation; EPO, erythropoietin; GSH, glutathione (reduced); HIF-1α, hypoxia inducible factor-1α; HO-1, heme oxygenase 1; ICAM-1, intracellular cell adhesion molecule 1; iNOS, inducible NOS (NOS2); IPC, ischemic preconditioning; NFκB, nuclear factor κB; NO, nitric oxide; Nrf2, nuclear factor (erythroid-derived 2)-like2; OSA, obstructive sleep apnea; ROS, reactive oxygen species; SOD, superoxide dismutase; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

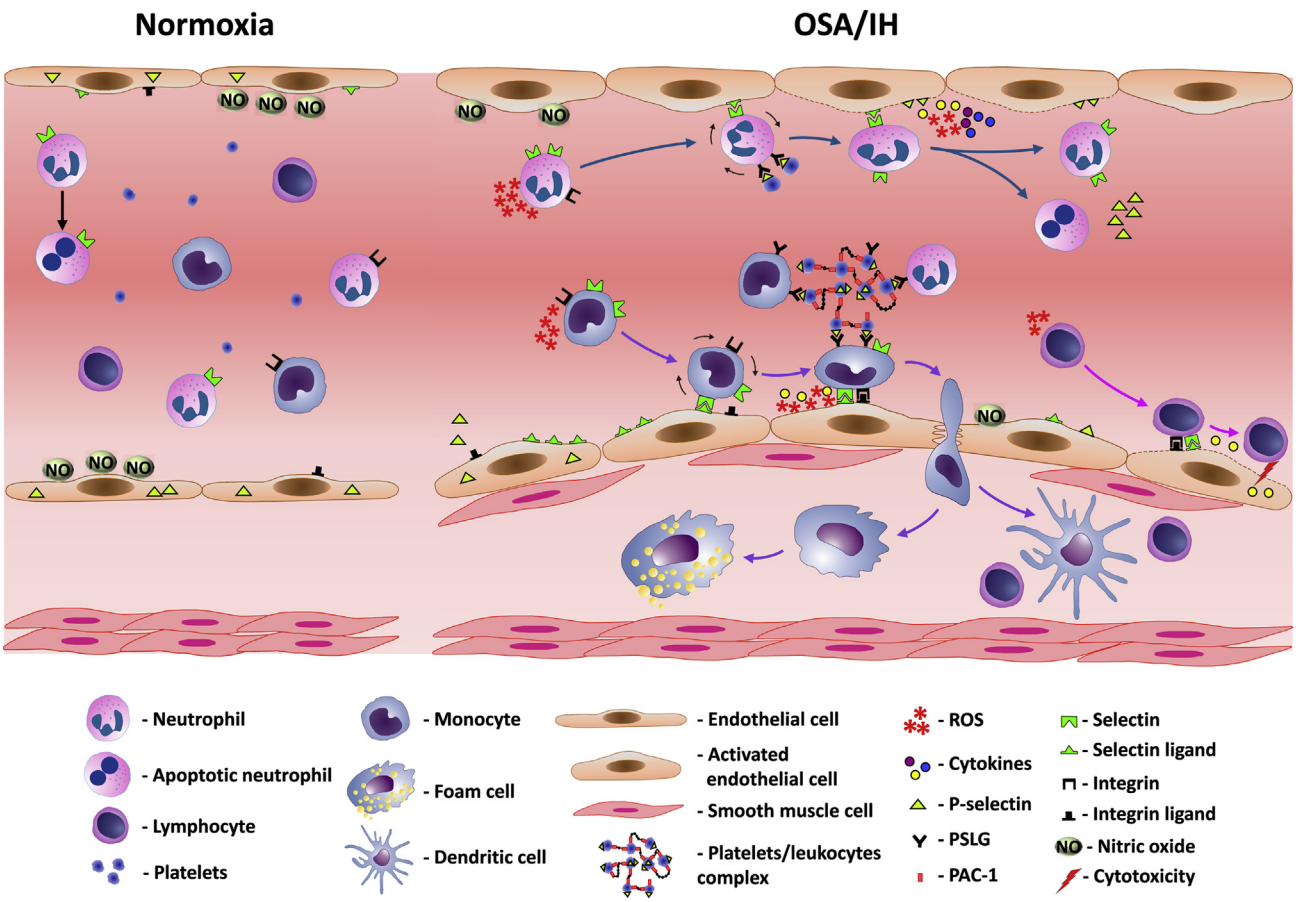

Fig. 2. Leukocytes endothelial cell interactions in OSA/IH, compared to normoxia. Neutrophils, monocytes, lymphocytes, platelets and endothelial cells are activated in obstructive sleep apnea/intermittent hypoxia, producing higher amounts of reactive oxygen species, adhesion molecules, and inflammatory cytokines and lower amounts of nitric oxide. These promote leukocytes/platelets/endothelial cells interactions, and induce endothelial cell injury. OSA/IH also promotes formation of foam cells and dendritic cells which further induce atherosclerosis.
IH, intermittent hypoxia; OSA, obstructive sleep apnea; ROS, reactive oxygen species; PAC-1, specific marker for glycoprotein (GP)IIb/IIIa; PSLG-1, P-selectin glycoprotein ligand 1.

studies which provided evidence implicating oxidative stress and inflammation in the development of endothelial dysfunction, atherosclerosis and cardiovascular morbidity in OSA (reviewed in [9,18]). Moreover, this sequence of events was further investigated and elaborated in recent years. By using animal models mimicking OSA and cell culture models of IH in vitro, the involvement of additional signaling molecules and transduction pathways such as p38 MAP kinase (p38 MAPK) and extracellular signal-regulated kinase (Erk1/2) was demonstrated [23,24]. In addition, much has been established on the involvement of ROS in fundamental mechanisms associated with OSA including sympathetic activation, and various cardiovascular risk factors (such as obesity, hypertension, hyperlipidemia, diabetes and insulin resistance), their intricate interactions with oxidative stress, and their contribution to the development of atherosclerosis and cardiovascular morbidity in patients with OSA [25]. However, in many of these co-morbidities it is not always clear whether oxidative stress or inflammation is the **cause** or the **consequence.** These intricate interactions between oxidative stress and the various co-morbidities associated with OSA are illustrated in Fig. 3A, and additional pathways involved in these interactions are described in Fig. 3B (see review in [9]). It should be noted that continued oxidative stress and the resultant inflammation which mediate most chronic diseases described in Fig. 3 were also shown to contribute to the initiation and progression of cancer [16], which is an emerging pathology associated with OSA [26,27], as well as in IH-treated rodents [28]. The involvement of ROS in tumorigenesis and metastasis is well established [15]. Thus, it remains to be seen whether IH-associated ROS contribute to the increased incidence of cancer and metastasis in OSA [29]. Yet, increased ROS and oxidative stress present in a vast number of OSA associated diseases on one hand, and IH-associated ROS on the other, make ROS a major contributor to OSA associated comorbidities.

ROS/RNS in physiology, pathophysiology and in OSA

In normal physiological conditions there is a homeostasis between the production of ROS and/or reactive nitrogen species (RNS) and the defense mechanisms eliminating them in order to control and maintain a tightly regulated redox (oxidation/reduction) balance for signaling pathways. This redox state is specific for each cell and determines its cellular function. Redox homeostasis is imperative in order to regulate proliferation, differentiation, and cell death (apoptosis) by modulating growth factors and transcription factors to maintain life (for review see [30]). However, various pathologies and conditions like I/R or inflammation which alter the tightly regulated redox balance result in oxidative/nitrosative stress and oxidant damage [13,18,30]. It is estimated that more than 100 human diseases and pathological conditions are associated with oxidative stress. Among them atherosclerosis, carcinogenesis and metastasis, cardio- and cerebro-vascular disease, diabetes, insulin resistance, hypertension, inflammatory diseases, and neurological

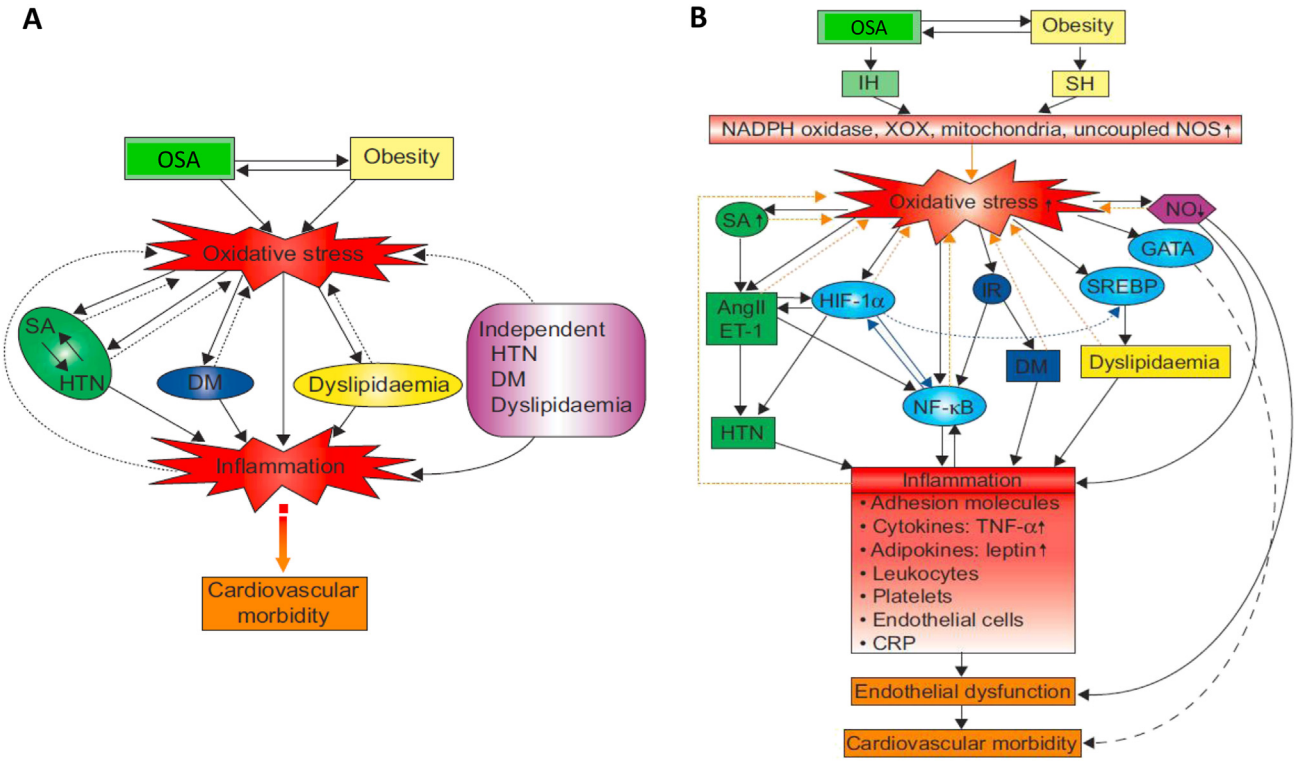

Fig. 3. Schematic illustrations suggestive of the central role played by oxidative stress and inflammation in OSA and the development of associated conditions and comorbidities. (**A**) Comorbidities such as sympathetic activation, hypertension, type 2 diabetes, dyperlipidemia insulin resistance or obesity can be induced by oxidative stress or develop independently. Once these comorbidities develop, regardless of the initiating factors, they elicit a series of intricate interactions with various transduction pathways, promoting oxidative stress and inflammation. The enhanced oxidative stress exacerbates inflammation, which in turn further exacerbates oxidative stress, generating a vicious cycle, eventually leading to cardiovascular morbidity. (**B**) A tentative model suggestive of oxidative stress as a unifying paradigm in OSA and the development of comorbidities that aggregate with OSA through upregulation and interactions of various transcription factors. Obesity, hypertension, inflammation, sympathetic activation, type 2 diabetes and dyslipidaemia, all have an oxidative stress component and thus interact with each other through ROS molecules and inflammation.
Ang, angiotensin; CRP, C-reactive protein; DM, type 2 diabetes; ET-1, endothelin-1; GATA, GATA transcription factor; HIF, hypoxia-inducible factor; HTN, hypertension; IH, intermittent hypoxia; IR, insulin resistance; NF, nuclear factor; NO, nitric oxide; NOS, nitric oxide synthase; OSA, obstructive sleep apnea; SA, sympathetic activation; SH, sustained hypoxia; SREBP, sterol regulatory element binding protein; TNF, tumor necrosis factor; XOX, xanthine oxidase; Orange dotted arrows: oxidative stress induced by the various conditions and comorbidities, further augmenting oxidative stress and, consequently, inflammation. (**A**) and (**B**) reproduced with permission of the European Respiratory Society. *Eur Respir J June 2009 33:1467-1484;* http://dx.doi.org/10.1183/09031936.00086608.

deficits and disorders such as Alzheimer and Parkinson disease as well as aging [13,14,30]. Thus, ROS and oxidative stress have come to occupy a major research avenue in health and disease. This is exemplified by the cumulative number of publications retrieved from the PubMed using the terms "oxidative stress" (>100,000) and "superoxide" (~80,000), showing a 4-fold increase over the last decade. The term "redox signaling" is also rapidly increasing in the last decade (>10,000) [31]. Based on this massive volume of publications, the understanding of IH associated redox biology can be further implemented in OSA/SDB.

Chemistry of ROS and RNS

ROS are atoms or molecules with one unpaired electron or more in their outer orbit, which makes them chemically unstable and reactive. Thus, by interacting with various biomolecules, ROS can cause damage by altering their structure and functions, thereby affecting the whole organism.

Endogenous ROS are constitutive by-products of normal oxygen metabolism, and are primarily generated by the respiratory chain of the mitochondria for signaling purposes. As such they serve to communicate and regulate cellular processes through mechanisms termed cell signaling or transduction pathways. Most of the oxygen consumed is utilized for energy metabolism. However, due to electron leakage at complex I and III, 1–3% of the oxygen consumed is converted into superoxide anion ($O_2^{\bullet -}$) instead of water. Additional sources of superoxide include production by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox), xanthine oxidase and dysfunctional or uncoupled nitric oxide synthase. Besides these endogenous sources, the organism might be also exposed to a variety of exogenous ROS/RNS sources including cigarette smoke, environmental pollutants, ionizing radiation and hypoxia, making antioxidant defense mechanisms vital.

Superoxide anion is the primary source of ROS and can give rise to oxidants like hydrogen peroxide (H_2O_2) which can generate additional ROS molecules including the very potent hydroxyl radical ($OH^{\bullet}$). Moreover, the presence of reduced transition metals such as Cu^{+} and Fe^{+2} (Fenton reaction) facilitates its production. In leukocytes, due to myeloperoxidase activity, also hypochlorous acid (HClO) is formed. The interaction of $O_2^{\bullet -}$ with NO, the product of nitric oxide synthase (NOS) gives rise to a highly potent oxidizing agent, namely, peroxynitrite ($OONO^{-}$) which can react and damage critical cellular biomolecules [32].

Effects of ROS/RNS on various biomolecules

The reactivity of ROS/RNS was shown to damage a great number of biomolecules including lipids, proteins, thiols, carbohydrates and DNA, as well as cellular components (Fig. 4). However, not all oxidative modifications are harmful and the outcome depends on a great number of factors.

Effects on lipids. Although superoxide is the primary and most abundant of ROS, hydroxyl radical ($OH^{\bullet}$) is the most potent of ROS, and lipids are the most sensitive molecules prone to oxidation. When hydroxyl radicals encounter lipids this results in lipid peroxidation and can also propagate lipid chain reactions [33]. The oxidation of lipids by $OH^{\bullet}$ can affect many physiological processes and contribute to cellular dysfunction and cardiovascular disease [34]. The most investigated end products assessing lipid peroxidation include malonaldehyde (MDA) which is also mutagenic in mammalian cells, 4-hydroxy-2-nonenal (HNA), thiobarbituric acid reactive substances (TBARs), (F2) 8-isoprostanes and oxidized low-density-lipoprotein (oxLDL). Oxidation of phospholipids or unsaturated fatty acids by NO derived oxidants such as peroxynitite also impacts on their functions and promotes atherogenesis, or anti-inflammatory properties, depending on the specific phospholipid [32].

Effects on DNA. The reaction of $OH^{\bullet}$ with DNA can damage purines, pyrimidines and deoxyribose, causing breaks and lesions which permanently damage the genetic material. Such DNA modifications can initiate mutagenesis and cancer development. In most studies, oxidation of DNA is assessed by the production of 8-hydroxyguanine (8-OH-G) [35]. Another important radical implicated in mutagenesis, inflammation, and cell death is $ONOO^{-}$ which can cause single and double DNA strand breaks [32].

Effects on proteins. Posttranslational protein modifications occur in order to help cells cope with environmental challenges and expand protein functions. Oxidative modifications of proteins by ROS and RNS can be classified as reversible or irreversible oxidations, many of which are involved in signaling processes beneficial in health and disease (reviewed in [36]). Thiol (SH) oxidation of proteins participates in redox regulation of protein functions by ROS and RNS, and can protect proteins undergoing oxidation from the permanently damaging effects of further oxidation (since SH groups undergo reversible oxidation) [37]. Oxidation of thiols can occur at the side chain of amino residues containing SH groups, such as cysteine and methionine and results in di-sulfide (S—S) bonds between proteins thiol-groups or low molecular weight thiol molecules such as in glutathione (GSH), which is a reversible reaction [38]. Such a protein modification has an important role in redox signaling cascades and cellular defense systems to protect from various stresses and insults [39]. Oxidation of GSH and thiol containing proteins by NO-derived oxidants such as $ONOO^{-}$ depletes antioxidant activity, promotes neuronal damage and impairs myocardial contractility [32].

Irreversible protein oxidation occurs by formation of carbonyls on several amino residues like arginine, histidine, and lysine. Carbonylation of proteins can be followed by western blot analysis [40]. Importantly, recent studies implicate protein carbonyls in signal transduction and in ischemic preconditioning against I/R injury [36]. Another type of irreversible oxidative protein modification is by formation of protein nitrosylation yielding 3-nitrotyrosine which is formed between RNS and protein tyrosine residues. However, this is a highly selective process and not all proteins or all tyrosines undergo nitrosylations [41]. Yet, although 3-nitrotyrosine was shown to have deleterious effects, it was also detected in normal physiological conditions. Protein oxidation by NO-derived oxidants impacts on many disease states including atherosclerosis, and cardiovascular morbidity. Moreover, protein oxidation may result in activation, inactivation, or gaining a new function, depending on the specific oxidative modification taking place [32]. An updated and comprehensive review on protein oxidative modifications and their implications to health and disease can be found in [36].

Collectively, oxidation by ROS/RNS is involved in signaling transduction pathways as well as in many cellular responses affecting disease states. The extent and the biological consequences are determined by various factors including the type of ROS/RNS produced, their concentrations, the duration of exposure to those ROS/RNS, and by the ability of the cells to counteract oxidative/nitrosative stress. Thus, the molecular alterations inflicted by ROS/RNS on cells may vary considerably, ranging from minimal and reversible changes to severe energy depletion, altered DNA integrity, mitochondrial dysfunction and possibly cell death [32].

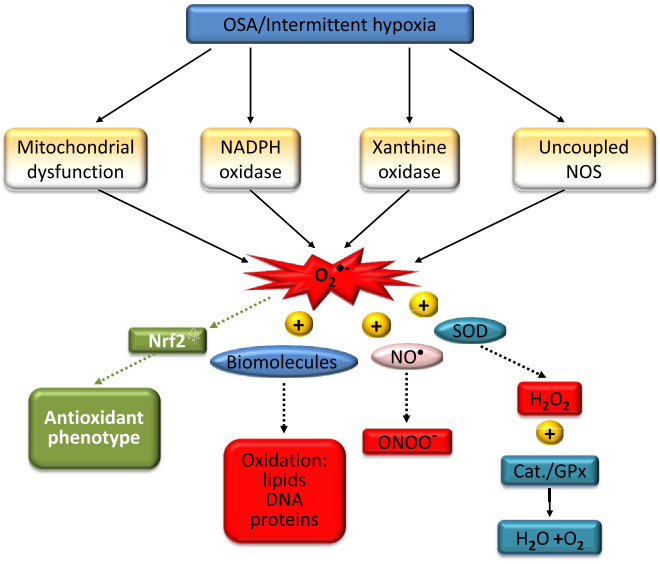

Fig. 4. The central role of superoxide and ROS in OSA and under intermittent hypoxia describing the main sources of superoxide, its interactions with biomolecules and antioxidant defense systems such as Nrf2. Superoxide oxidizes biomolecules. A reaction with NO facilitates peroxynitrite formation. Superoxide dismutase sequestrates the superoxide yielding hydrogen peroxide, while catalase and glutathione peroxidase may convert hydrogen peroxide to water and oxygen (equations are not balanced). Cat., catalase; GPx, glutathione peroxidase; H_2O_2, hydrogen peroxide; NO, nitric oxide; Nrf2, nuclear factor (erythroid-derived 2)-like2; $O_2^{\bullet-}$, superoxide anion; OSA, obstructive sleep apnea; ROS, reactive oxygen species; SOD, superoxide dismutase.

Circulating biomolecules as oxidative stress markers in OSA

The number of studies implicating oxidative stress in OSA patients in the last decade is overwhelming. The majority of these studies provide indirect evidence by using various markers; however, in a small number of studies also negative results were reported. Most oxidative stress/antioxidant markers were identified in the circulation and in bodily fluids such as the urine and saliva. In earlier studies, lipid peroxidation markers in plasma and serum were most frequently used [22,42,43], and were identified in exhaled breath as well [44]. Also, the levels of circulating oxLDL were shown to increase in patients with OSA [45]. More recent studies further confirmed and expanded these findings. Increased oxidative stress and lipid peroxidation in OSA was also associated with an increase in carotid intima media thickness [46], and endothelial dysfunction [47,48]. Oxidative stress in OSA was also associated with hypertension via the renin-angiotensin system [49,50], likely by activating Nox [51,52]. Treatment with nasal continuous positive airway pressure (nCPAP) or dental device attenuated these oxidative stress markers and improved endothelial function [22,42,44,47,50].

The oxidation of DNA in OSA was demonstrated by increased urinary excretion and plasma levels of 8-hydroxy-2'deoxyguanosine [48,53], while nCPAP treatment attenuated its levels [48]. Protein oxidation of carbonyls in plasma and erythrocytes of OSA patients was indicated in a number of recent studies, and was positively correlated with AHI [54–56]. Also high 3-nitrotyrosin

expression was demonstrated in endothelial cells [57], as well as in plasma [58], and was decreased by treatment with nCPAP [59].

Sources and targets of ROS/RNS: implications to IH and OSA

Mitochondria

There is substantial evidence to suggest that indeed ROS molecules are increased in patients with OSA and in response to IH by various sources including Nox, xanthine oxidase and NOS, while studies on mitochondria are limited.

Mitochondria are principal regulators of cellular integrity and function. As such, impaired mitochondrial function affects a wide range of diseases. Mitochondrial dysfunction is the major source of ROS/RNS notably during I/R associated morbidities. Thus, mitochondria are considered as a potential target for cardioprotection in acute myocardial ischemia [60].

Mitochondria in OSA and in animal models of OSA

Studies conducted on cells in culture, animal models mimicking OSA, as well as in patients with OSA demonstrate that IH induces mitochondrial dysfunction, thereby increasing oxidative stress [61–65]. Moreover, abnormal mitochondrial organization and oxidative activity was shown in the palate muscles of OSA patients [66]. In mice, mitochondrial ROS generated by chronic IH impaired pancreatic β-cell function indicating the potential contribution of mitochondrial ROS to the development of type 2 diabetes [67]. Also, neuronal death in neonatal mice treated with chronic IH/chronic hypercapnia was shown to result from mitochondrial dysfunction and increased ROS [68]. Similarly, in cultured neuronal cells in vitro, mitochondrial ROS was increased, while overproduction of superoxide dismutase (MnSOD) – the superoxide quenching enzyme in mitochondria – protected against IH-mediated oxidative stress [64,69]. Additionally, the levels of mitochondrial ROS were dependent on the severity of the IH in a model of focal cerebral ischemic injury, in which the IH was applied prior to the ischemic injury. Importantly, depending on the severity of the IH, mitochondrial ROS levels were also associated with both beneficial (producing low ROS levels) as well as detrimental (producing high ROS levels) effects on the brain [70]. Although most of these studies suggest that mitochondrial ROS and mitochondrial dysfunction in OSA/IH are increased, the reports are still limited and incomplete. Thus, the dichotomous IH-severity dependent mitochondrial ROS levels and their associated dichotomous effects on affected organs such as the heart and brain should be further explored [70].

NADPH oxidase

NADPH oxidase (Nox) is a very complex and ubiquitous enzyme expressing multiple functions in a variety of cells and tissues and comprising seven family members (Nox1-5, Duox1, and Duox2). Nox generate $O_2^{\bullet-}$ by transferring an electron from NADPH to molecular oxygen which is a tightly regulated enzymatic activity. This multicomplex enzyme consists of membrane-bound Nox subunits and p22phox (which is highly expressed throughout the cardiovascular system) and cytosolic subunits (p47phox, p67phox, p40 phox) and is activated upon assembly. The Nox2 subunit that serves as the electron transfer (formerly, gp91phox)-is composed of flavocytochrome b558, and the first to be discovered, is the primary ROS producing enzyme in leukocytes. The actively assembled Nox2 generates $O_2^{\bullet-}$ in response to various invading microorganisms in order to combat and eliminate infections. It also generates ROS in response to soluble or particulate stimuli and as such it is considered beneficial to the host [71]. However, if activated by I/R it can cause damage to surrounding tissues.

Besides its major role in immune defense, Nox2 is also expressed in a variety of other tissues. It was shown in the central nervous system (CNS), blood vessels, hematopoietic stem cells, endothelial cells, cardiomyocytes and throughout the cardiovascular system, where it is expressed at a much lower activity and generates lower amounts of ROS for signaling functions (reviewed in [51]). In the cardiovascular system, Nox2 primarily regulates endothelial and cardiac functions. It is activated by numerous vasoactive stimuli including adenosine A2 receptors and angiotensin II, and therefore can contribute to hypertension [52]. Because of its pattern of expression in the cardiovascular system it is also implicated in controlling vessel tone, endothelial cell proliferation, migration, angiogenesis, and cardiac hypertrophy and remodeling [51]. Nox2 is also implicated in a great number of pathologies including gastrointestinal inflammation, myocardial injury, restenosis after angioplasty, melanoma, diabetes and neurodegenerative diseases, and in hypertension, as indicated earlier [72]. The involvement of Nox2 from phagocytic cells was also recently described in the setting of oxidative injury in ischemic stroke; ROS released from resident and recruited phagocytes were suggested to promote carotid plaque rupture and enhance cerebral ischemic injury in stroke. Selectively targeting neurotoxic ROS and increasing neuroprotective oxidants have recently produced promising results [73].

Other Nox isoforms slightly differ in subunit organization and in function. For instance, Nox1 is expressed in endothelial cells, vascular smooth muscle cells, neurons, astrocytes and microglia. This isoform is also implicated in various pathologies including colon and prostate cancer, gastrointestinal inflammation, hypertension and restenosis after angioplasty. Additional isoforms; Nox3, Nox4, Nox5 and Duox1 (Nox6), Duox2 (Nox7) are described elsewhere in detail [51,72,74].

Interestingly, recent data indicate that Nox4 derived ROS, which exist in the immediate environment of the nucleus, when deregulated can cause genomic instability and induce oncogenic DNA damage, thus, promoting carcinogenesis. However, Nox4 in the nucleus does not act solely as a threat, since ROS also mediate signal transduction in normal cells by regulating redox sensitive specific cysteine residues in specific effector proteins [75].

An emerging concept in Nox generated ROS indicates that ROS also regulate the functions of immune cells infiltrating the tumor environment and stimulate angiogenesis by macrophages and regulatory T cells [76]. The evolving understanding that the Nox family of enzymes is a major source of elevated ROS levels and plays a pivotal role in the initiation or progression of a multitude of diseases, has led to intensive research towards identifying selective Nox inhibitors for therapeutic purposes in a wide array of diseases associated with increased Nox levels [73,74], this, in order to inhibit the production of ROS rather than eliminating them after being produced. Thus, the role of Nox as generating ROS for signaling in health is undisputable [51,77], along with its protective functions in infectious and inflammatory conditions to restore health, and the hazards of its activation by I/R.

NADPH oxidase in OSA and in animal models of OSA

NADPH oxidase is by far the most studied source of ROS in OSA, and was mainly described in rodent models mimicking OSA. In earlier studies the activity of Nox was shown to increase in tissues such as the brain and the carotid body [78–80]. Recently, however, the evidence on the impact of Nox2 associated oxidative stress in IH/OSA is mounting. Nox2 was shown to participate in neurobehavioral and cognitive impairments [81,82], and in adverse cognitive effects observed in IH treated mice fed high fat diet [83]. Nox2 was also shown to increase myocardial susceptibility to infarction in IH treated rats [84], and this was correlated with the severity of IH [85]. Erectile dysfunction in long term IH treated rats was also attributed to Nox2 [86], and chronic-IH induced arterial

hypertension in mice was facilitated by Nox2 [87]. Moreover, the severity of Nox2-dependent oxidative stress was also shown to have organ specific effects [88].

Unlike animal studies, the studies investigating Nox2 in OSA patients are limited. However it is obvious that Nox2 is an important oxidative stress contributor in humans and is associated with IH. Earlier studies indicated indirectly that Nox2 is activated in patients with OSA by demonstrating that ROS production was increased in activated neutrophils and monocytes [17,20,21]. Thereafter, two recent studies in children and adult patients with OSA emphasized the importance of Nox2 polymorphisms to ROS production [89,90]. Pierola et al. analyzed blood samples of OSA patients and controls for Nox2 activity and distribution of allelic frequencies, and their possible relationship with oxidative stress levels using 8-isoprostane as a marker. Their data suggest that gene polymorphism at the p22phox subunit of Nox2 affects the development of oxidative stress in OSA as compared to controls [90]. Also, in a study by Gozal et al. investigating children with OSA, Nox gene polymorphism at p22phox subunit was an important component which contributed to cognitive functions and deficits. Nox activity and the oxidative stress marker, urinary 8-hydroxydeoxyguanine, were lower in controls and among children with OSA without cognitive deficits compared to OSA with cognitive deficits, and were also associated with a specific gene polymorphism [89]. Although the studies on Nox in OSA are limited, likely due to technical and ethical constraints, it is clear that its activity is increased in OSA and contributes to oxidative stress. However, it is not clear how this may affect its signaling functions.

Xanthine oxidase

Xanthine oxidoreductase is another superoxide producing enzyme. It was discovered nearly 30 years ago as the critical enzyme generating ROS in I/R injury [91]. The enzyme is ubiquitously expressed in various tissues including the vasculature, plasma and the liver. It catalyzes purine degradation by oxidizing hypoxanthine to xanthine and subsequently to uric acid [11]. During ischemia, the enzyme is activated by action of proteases converting it to xanthine oxidase. Thus, during reperfusion, when tissue oxygenation is restored, the activated xanthine oxidase utilizes hypoxanthine and oxygen to produce superoxide and ROS in the form of H_2O_2 and hydroxyl radical [11,91].

Multiple inflammatory, cardiovascular and cardiopulmonary diseases are associated with xanthine oxidase activity. These include heart failure, chronic obstructive pulmonary disease (COPD), pulmonary hypertension and diabetes type I and II. Moreover, recent evidence suggests that xanthine oxidase is also able to act as nitrate/nitrite reductase. Thereby it may have a critical role in producing NO under ischemic/hypoxic conditions which in fact limit the functional capacity of nitric oxide synthase [92]. Thus, in stressful hypoxic conditions, xanthine oxidase could also act in a beneficial way. However, generating ROS vs. NO by xanthine oxidase depends upon key micro-environmental factors such as decreased oxygen availability, or inflammatory conditions, whose interplay impacts on the type of the reactive species (ROS vs. NO) generated. This new function of xanthine oxidase activity sheds a new light on this enzyme as a crucial component in the maintenance of redox homeostasis [92].

Xanthine oxidase in OSA and in animal models of OSA

Xanthine oxidase is another potential source of ROS with implications to OSA morbidity. Most of the studies, to date, present indirect evidence for its activation in OSA and in animal models mimicking OSA. For instance, by treating OSA patients with a specific xanthine oxidase inhibitor – allopurinol – plasma lipid peroxidation by-products such as MDA were lowered and endothelial function was improved, consistent with the decrease in oxidative stress [93]. In that context, uric acid – the xanthine oxidase metabolic by-product was shown to increase in plasma of OSA patients [11,94]. In more recent studies, treatment of rats with chronic IH and allopurinol, confirmed these findings [95], and further established that the use of the xanthine oxidase inhibitor besides improving oxidative stress also improved myocardial dysfunction and myocyte apoptosis [96]. Also, a study conducted on the cell line pheochromocytoma PC12-provided direct evidence on xanthine oxidase activation in response to IH, and further established its involvement in HIF-2α degradation due to IH [97]. Collectively, these studies confirm the involvement of xanthine oxidase in contributing to oxidative stress in OSA.

Nitric oxide and nitric oxide synthase (NOS)

Nitric oxide is a weak, soluble, and highly diffusible gas functioning as a signaling molecule. NO acting through cyclic GMP (cGMP), is primarily known for its endothelial-derived relaxing properties on smooth muscle cells, as well as a neurotransmitter [32,98]. But it is also known for its cytotoxic properties in macrophages against various microorganisms in inflammatory-immunological conditions. NO's signaling and protective properties occur at low nanomolar concentrations. Conversely, its cytotoxic and anti-inflammatory activity which is beneficial to the host is performed at the micromolar range [32,99].

NO is synthesized by three NOS isoforms, by oxidation of the terminal guanido group in L-arginine, in the presence of molecular oxygen, and tetrahydrobiopterin (BH_4) and NADPH as co-factors. If however, substrates or co-factors are lacking or present at low concentrations, instead of producing NO, NOS become dysfunctional and produce $O_2^{\bullet-}$. This altered function of NOS is termed **NOS uncoupling** [100]. Of the three NOS isoforms, neuronal NOS (nNOS, NOS1) was initially identified in the brain but is also found in a number of other cell types including the myocardium. Endothelial NOS (eNOS, NOS3) was initially identified in the endothelium but is also expressed in cardiomyocytes. Both are calcium-calmodulin dependent and are expressed constitutively at lower levels. NOS2, on the other hand, is an inducible (iNOS), calcium-independent enzyme, that is regulated by NFκB and activated by inflammatory mediators such as cytokines and bacterial products. When activated it can produce 100-fold higher NO levels than the constitutive isoforms [32]. In addition there is a negative interaction between the inducible and the constitutive isoforms in which iNOS activity might be impaired by nNOS [101].

Under physiological conditions the eNOS which produces NO in the vasculature acts as an anti-hypertensive and anti-thrombotic factor. It relaxes vascular smooth muscle cells, inhibits leukocyte adhesion, and platelet aggregation and adhesion to endothelial cells in the vascular wall (Fig. 2). Therefore, acting as an anti-atherosclerotic factor (reviewed in [100,102]). However, in atherosclerosis or in diseased vessels, the availability of NO decreases and the normal physiologic anti-atherogenic and anti-thrombogenic properties of NO are diminished. This decrease in NO can result from inactivation by superoxide or by uncoupling of NOS.

In the heart, under normal physiological conditions, NO is produced by the two constitutive isoforms, eNOS and nNOS, which are located at different intracellular sites in ventricular myocytes. The levels of NO production in myocytes are low. NO modulates heart function and regulates contractility and growth. Altered functions of NOS by oxidative stress or lack of substrates or co-factors contribute to heart failure by causing irregular contractile function [103]. The inducible iNOS is not normally expressed in myocytes, unless induced during inflammation which occurs in heart failure. Once iNOS is expressed, it produces high NO levels leading to cardiac dysfunction [104]. However, iNOS is essential in

inflammatory conditions for killing bacteria and other microorganisms by producing NO and superoxide which upon interaction produce toxic peroxynitrite molecules [105]. On the other hand, since NO is also a potent vasodilator released by endothelial cells to maintain endothelial function, as well as a neurotransmitter, neutralizing its activity by $O_2^{-\bullet}$ can propagate nitrosative stress endothelial dysfunction and neurological deficits. Thus, maintaining a delicate balance between ROS and RNS is unpredictable in stressful conditions and is difficult to isolate the "good" from the "bad/ugly".

In many clinical studies NO levels are assessed in plasma or serum by determination of nitrite/nitrate, the end products of NOS. In such a setup the specific isoform producing NO cannot be identified, nor its intracellular localization or whether the values obtained result from nitrate rich diets. Moreover, because of nitrate rich diet or the negative interaction between the constitutive and the inducible forms, which in many instances are expressed at the same cell, NO values determined in the circulation could give erroneous values as to the state of NO production in the clinical setting.

Nitric oxide and nitric oxide synthase in OSA

In earlier studies, indirect evidence was presented on the involvement of NOS in OSA, based on its vasoactive properties on endothelial function [5]. Also, circulating NO levels were shown to decrease in OSA, by analyzing nitrite/nitrate levels [106–108]. Although it was not clear which of the NOS isoforms produced NO and what was the cellular origin, it was presumed that circulating NO levels were mostly produced by eNOS from endothelial cells [5,11]. Treatment with nCPAP was shown to increase circulating NO levels [106–108], as well as the levels of its substrate L-arginine [108]. Interestingly, in a recent study conducted by Kheirandish-Gozal et al. [109], NO production by monocytes was shown to differentiate between OSA children with and without endothelial dysfunction. While in children with OSA and endothelial dysfunction NO levels were low, in children with OSA without endothelial dysfunction NO levels were comparable to controls.

The association between eNOS expression in endothelial cells and endothelial function was described in a number of studies. Jelic et al. [57] have shown that oxidative stress was increased in endothelial cells harvested from the circulation of patients with OSA by the presence of higher amounts of 3-nitrotyrosine compared with endothelial cells from controls. But also eNOS levels were attenuated, while iNOS levels were increased in that setting. These changes were also associated with decreased flow mediated dilation in OSA. In patients adhering to nCPAP, eNOS expression was increased while that of iNOS was decreased, and endothelial function was improved (patients studied were mild-moderate OSA with an average AHI = 25 and ODI4 = 12). More recently Patt et al. [110] had shown that peroxynitrite deposits and eNOS transcription were increased, while endothelial function was decreased in the microcirculation of patients with OSA (average AHI = 35). The increased peroxynitrite is indicative of overproduction of NO as well as superoxide, since it is a by-product of their interaction. Peroxynitrite is also a good marker of oxidative stress. Thus, its presence may suggest decreased NO bioavailability due to the interaction with the increased levels of superoxide. Increased overproduction of NO was also supported by the increased transcription of eNOS. In addition the antioxidant enzyme superoxide dismutase (SOD-1) was also upregulated, thus confirming antioxidant overproduction in the endothelium. Treatment with nCPAP attenuated the increases observed in peroxyntrite deposits and the transcription of eNOS, and SOD-1. In a very thorough and recent study by Kaczmarek et al. [111], vascular dysfunction in OSA patients was investigated and was also confirmed in mice exposed to IH using molecular biomarkers. These authors showed that the relative expression of eNOS mRNA from skin biopsies of OSA patients was decreased in mild patients (oxygen nadir >75%, average 80 ± 6.0%) as compared to controls. However, in severe patients (oxygen nadir < 75%, average 65.0 ± 3.8%), eNOS mRNA was slightly higher or similar to controls. These apparently contradictory findings may indicate that eNOS activity in severe OSA might be increased as compared to mild OSA due to increased inflammation and the complex interactions between various signaling cascades. This assumption is corroborated by the similar increases observed in the relative RNA expression of the adhesion molecule VCAM-1 (vascular cell adhesion molecule l) as well as that of HIF-1α and its downstream gene – vascular endothelial growth factor (VEGF) – in the severe patients. While the studies by Jelic et al. [57] and Patt et al. [110] are seemingly contradictory with regard to eNOS activity, the latter findings by Kaczmarek et al. concur with both studies, by showing a decrease or an increase in eNOS compared to controls, depending on the severity of the hypoxemia [111]. Moreover, while low eNOS activity was associated in Jelic et al. study with endothelial dysfunction, high eNOS activity was associated with more severe endothelial dysfunction in Kaczmarek et al. study [111]. However, such diverse findings between the studies could have also resulted from differences in tissue specificity. Altogether, these findings indicate the importance of the NO/superoxide ratio of NOS activity to vascular function. These last studies based on cellular and molecular measures attest to the complexity of the interactions between ROS/RNS/NO and endothelial dysfunction in OSA. Additionally, they clearly point to the importance of the severity of OSA in modulating NOS activity and local microcirculatory oxidative status. Moreover, recent studies investigating NOS polymorphisms and epigenetics in children further demonstrate the complexity and significance of differences in eNOS expression associated with the dichotomous phenotypes of endothelial function in OSA [109,112,113].

Antioxidant defense mechanisms in hypoxia and reperfusion

Antioxidant defense mechanisms have evolved to counteract excessive endogenous or exogenous ROS/RNS and protect cells and tissues from their damaging actions. These include a number of enzymatic systems as well as non-enzymatic antioxidant molecules and the transcription factor nuclear factor (erythroid-derived 2)-like2 (Nrf2) which acts as a master regulator of antioxidant responses [14,30].

Superoxide dismutase (SOD), is the primary enzyme catalyzing ROS by dismutating superoxide and producing H_2O_2 and oxygen (Fig. 4). SOD has three isoforms; the cytosolic (Cu–Zn-SOD, SOD1) which is constitutive, the mitochondrial SOD (Mn-SOD, SOD2) and the extracellular type (SOD3). The mitochondrial SOD is an inducible form and the one protecting from oxidative stress and during I/R injury. It is by far the most protective isoform for eliminating ROS [114]. The H_2O_2 produced by SOD is converted by catalase and glutathione peroxidase (GPx) to water (Fig. 4). Small non-enzymatic molecules acting as antioxidants include vitamins C and E, flavonoids, carotenoids, polyphenols and the tripeptide glutathione (GSH). From the smaller antioxidant molecules, GSH is the most abundant and immediate intracellular antioxidant. GSH is a co-factor for many detoxifying enzymes against oxidative stress. It holds many antioxidant functions, such as scavenging hydroxyl radicals, and eliminating H_2O_2 and lipid peroxides. Its thiol group (SH) acts to reduce disulfide bonds in cytoplasmatic proteins while 2GSH molecules are oxidized in the process and converted to glutathione disulfide (GSSG). This is a reversible process and the oxidized form can be reduced back to its reduced GSH form. In the

nucleus GSH maintains the redox state of proteins crucial for DNA repair. The 2GSH/GSSG ratio is a good indicator of the cellular oxidative stress [30].

Another very important thiol dependent antioxidant defense system is thioredoxin. Thioredoxins are small molecular enzyme reductases which catalyze disulfide/dithiol (S–S/SH) changes. The system includes NADPH, thioredoxin reductase and thioredoxin. The thioredoxins function as an antioxidant system by regulating protein and DNA repair and the activity of many redox sensitive transcription factors. Thus, both systems, thioredoxin and glutathione control the cellular redox [115].

In recent years, however, it has become increasingly evident that antioxidant defense mechanisms are controlled by an anti-oxidant transcription factor. Namely, Nrf2, which is a master regulator of detoxification processes that mitigate oxidative stress. Nrf2 is constitutively expressed in most cells and tissues but primarily in the liver where routine detoxification reactions occur, by controlling phase II detoxifying enzymes. In basal conditions, Nrf2 is sequestrated in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1). Apart from the phase II detoxifying enzymes, Nrf2 regulates a great number of other genes which attenuate oxidative stress, including those involved in glutathione synthesis, SOD, stress proteins like heme oxygenase (OH-1), and hundreds of genes associated with survival. These are classified as antioxidant enzymes due to their role in redox balance and thiol homeostasis [14].

Nrf2 is activated by ROS and I/R during reoxygenation and mediates cytoprotective gene expression in I/R injury. This was shown by attenuating ROS production during reoxygenation using the antioxidant N-acetylcysteine (NAC) which also inhibited Nrf2 activation [116]. More recent studies clearly demonstrate that Nrf2 functions as a protective mechanism for cardiomyocyte survival during hypoxia and I/R [117]. These findings indicate that reoxygenation-dependent Nrf2 activity facilitates protection during ischemia (ischemic preconditioning) through the induction of antioxidant gene expression and that ROS may be critical in signaling this event, and thus, attenuating cardiovascular disease [118]. It is indicated that inhibition of thioredoxin triggers Nrf2 activation by directly regulating its inhibitor Keap1. Moreover, besides Nrf2, the activity of many other redox-sensitive transcription factors such as NFκB and HIF-1α are also mediated by thioredoxin or GSH [115].

Antioxidant pathways in OSA and IH

Diminished antioxidant capacities, which alter the tightly regulated redox balance and contribute to oxidative stress, were also described in OSA. Total anti-oxidant capacity in OSA was reported to decrease in a number of studies and lowered levels of vitamins A and E, and SOD were described as well [43,119–121]. Additionally, the ability of serum albumin to act as an anti-oxidant was impaired in OSA and was improved after nCPAP treatment [122]. Also, thiol levels [120] and paraxonase-1(PON-1) activity which serves to protect high-density lipoprotein (HDL) and LDL from oxidation were lower in OSA [42,120]. This finding is in line with the increases observed in oxLDL levels and the appearance of dysfunctional HDL in OSA [45]. It should be also noted that PON-1 activity was significantly negatively correlated with the severity of the syndrome but not with age or BMI (Lavie L. & Lavie P. unpublished observations). Interestingly, a gene microarray study performed on blood cells of OSA patients indicated that the oxidative stress modulating genes HO-1, SOD1, SOD2, and catalase were altered, further supporting a role for oxidative metabolism in OSA [123].

Additional protective mechanisms were also reported in OSA. For instance, the levels of thioredoxin, the thiol regulating protein, were determined in plasma of patients with severe OSA before and after nCPAP and compared to non-OSA controls. Thioredoxin levels were significantly higher in OSA than in non-OSA controls. After a month treatment with nCPAP, thioredoxin was significantly decreased. Thioredoxin was also significantly positively correlated with AHI and the percentage of time spent with SaO_2 <90% [124]. These findings were recently replicated demonstrating a negative correlation with lowest O_2 saturation and confirming the positive correlation with AHI [125]. Also, in a rat model treated with IH, increased thioredoxin mRNA levels were reported in myocardial tissues [85]. In these studies described above, the authors concluded that thioredoxin is a good oxidative stress marker. However, given the protective role of thioredoxin, these findings clearly support the notion that protective mechanisms are upregulated due to IH and in OSA in a severity dependent manner, along with the increases noted in oxidative stress. These studies suggest that protective mechanisms are upregulated in order to rebalance the redox state.

Importantly, dichotomic tissue specific effects were also reported in response to IH. While exposure of rats to chronic IH caused systemic oxidative and inflammatory responses, as is well established, cardio-protective antioxidant and antiinflammatory mechanisms were identified in the heart. Hence, cardiac MDA, TNF-α and IL-6 were lower in hearts of chronic IH-treated rats as compared to control rats. Chronic IH also upregulated protective functions such as Nrf2 and HO-1. However, despite the increase in such a local protective compensatory mechanism, cardiac damage was also observed [126]. Thus, the overwhelming number of studies reporting positive findings with a multitude of enzymes and markers as specified above clearly attest to the induction of antioxidant protective mechanisms in a severity dependent manner alongside with the increase in oxidative stress in OSA. Likely, when “bad” damaging molecules are increased, “good” protective molecules are increased as well in order to counteract their negative consequences. It should be recognized however, that the changes described above are cell and tissue specific and are also affected by many additional factors such as the micro-environment, individual responses to IH, and the severity of the IH. Hence, the end result will depend on the balance between pro- and antioxidant activities.

Disease treatment with antioxidants – maintaining a delicate balance

Since redox homeostasis is crucial for maintaining health, one of the approaches undertaken to mitigate oxidative stress associated morbidities is based on intake of antioxidants [127]. However, such a treatment is not always successful. There is an ongoing controversy regarding treatment with antioxidants in clinical trials as an effective strategy to prevent cardiovascular events or other cardiovascular related morbidities as well as other morbidities, since the findings are inconclusive [128,129]. Likely, such inconclusive findings result from the multifaceted nature of ROS/RNS functions in health and disease. Antioxidant treatment may not necessarily be effective in all instances because of a great number of factors. It may depend on ROS/RNS quantities and sources, their intra/extracellular sites of action, the interactions with other ROS/RNS, cells/tissues specific differences, and genetic variation. The interplay between all these factors and the final balance in each cell/tissue will affect the outcome. Thus, the complex interactions between ROS/RNS/antioxidants need to be specifically elucidated in order to target a particular co-morbidity, or even to determine if a given individual should be treated by antioxidants, perhaps by developing specific genetic based assays, for use in personalized medicine. It should be recognized that intake of non-specific

antioxidants, such as vitamin E, or NAC does not neutralize all ROS produced and cannot reverse oxidative stress that has already occurred in vivo. Furthermore, because of the heterogeneity of ROS sources and the polymorphism of the enzymes producing them, decreasing the concentrations of some ROS by antioxidants does not imply that all types of ROS might be eliminated. Therefore, inhibiting the systems producing ROS, rather than scavenging ROS after being produced, could be a more effective strategy to prevent cardiovascular and other oxidative stress associated morbidities. Such an approach was undertaken in targeting NADPH oxidases and xanthine oxidase in vascular disease by using specific inhibitors for therapeutic purposes [74,93]. However, using a specific inhibitor can also disrupt the delicate cellular redox balance and consequently may inhibit vital signaling pathways essential for survival, resulting in detrimental rather than therapeutic effects. The dose, timing, and delivery of antioxidant treatment are very crucial as well, and intake of antioxidants might act sometimes as "too much of a good thing," disrupting cell homeostasis and adaptation to stress. For instance, antioxidant supplementation to lung cancer bearing mice accelerated its progression. Although oxidative stress and DNA damage were reduced, at the same time, antioxidant treatment also reduced the expression of p53, a key tumor suppressor protein [130]. Similarly, treatment with antioxidants in the intensive care unit was recently challenged due to inconsistent findings [47,48,131].

Antioxidant treatment in OSA was not systematically explored thus far. However, in a study by Grebe et al. [132], endothelial function was improved by treatment of OSA patients with a single intravenous injection of vitamin C. Unlike Grebe et al., in a small scale double blind crossover design, 17 OSA patients were divided into two groups and were treated for three months with antioxidant vitamins. Although vitamin levels in the plasma were increased after three months of treatment, limited improvement was noted in oxidative stress markers and endothelial function [133]. Much larger long term studies are needed to examine the effect of antioxidant treatment on OSA.

Protective mechanisms in morbidities associated with I/R

Coronary artery disease, which often results in acute myocardial infarction (AMI), and stroke are two debilitating/fatal diseases and among the leading causes of death worldwide. In both, the prevalence of OSA is higher than 60% [6,7,134–137] and the detrimental effects in both are largely attributed to I/R injury and the associated oxidative stress [138]. Acute myocardial ischemia and infarction can develop when coronary arteries are partially or completely occluded, reducing oxygen supply and nutrients. This may lead to cell death [139–141]. Stroke as well occurs when cerebral blood flow is interrupted, depriving oxygen and glucose supply to the brain. As a result, the affected area in the brain is impaired or becomes dysfunctional. Evidently, more than 80% of the cases account for ischemic strokes by occluding a cerebral artery, while 15–20% of all cases are hemorrhagic and result from rupture of a cerebral blood vessel [142].

Although complex, both AMI and ischemic stroke share many common pathophysiological mechanisms due to partial or complete occlusion of arteries. Mitochondrial dysfunction, increased generation of ROS and inflammatory cell activation are noted in both.

It is well established that high levels of ROS/RNS, affect cell viability and function by modifying proteins, lipids and genes. In the heart the increased production of ROS/RNS associated with I/R is manifested by contractile dysfunction and cell death. Conversely, ROS/RNS signaling can contribute to cardioprotection by activating various ROS/RNS dependent signaling pathways such as ischemic preconditioning (IPC).

Ischemic conditioning and potential implications for OSA/SDB

Ischemic preconditioning (IPC) is a well-established indispensable treatment for acute myocardial infarction [143]. IPC is in fact an experimental strategy in which the heart can be protected from an episode of acute lethal I/R injury by applying brief, repetitive non-lethal, episodes of ischemia (each lasting 2–5 min), followed by episodes of reperfusion (2–5 min) **prior** to a longer duration of potentially lethal sustained ischemia. This heart's own self-preserving mechanism can also act as **remote preconditioning** when either applied to the heart itself or to an organ or tissue that is remote from the heart. In most models of preconditioning investigated infarct size and ventricular arrhythmias are decreased [144]. Another form of protective effect is **post-conditioning**. This paradigm consists of series of brief occlusions and reperfusions of blood vessels which are applied at the transition period from the sustained ischemia to reperfusion. The post-conditioning effect is considered an applicable candidate in the clinical setting, at the onset of reperfusion [144].

Historically, the first study employing IPC protocol was published by Murry et al., in 1986 [145]. These authors established that intermittent periods of ischemia and reperfusion contribute to delaying lethal cell injury in the ischemic myocardium. The preconditioning protocol was applied to a canine model undergoing four 5-min circumflex coronary occlusions, each separated by 5-min of reperfusion. Thereafter, a sustained 40 min occlusion was applied, followed by four days reperfusion. Control dogs underwent the same protocol with a single 40 min occlusion but without the preconditioning phase as illustrated in Fig. 5A. Paradoxically, in the IPC treated dogs, after four days, ATP depletion was delayed, the intracellular structure was maintained and infarct size was reduced to 25% of its size in the control animals (as in Fig. 5B). The authors concluded that "these results suggest that the multiple anginal episodes that often precede myocardial infarction in man may delay cell death after coronary occlusion and thereby allow for greater salvage of myocardium through reperfusion therapy". Thus far, this study has been cited over 4300 times in PubMed. In subsequent studies with AMI patients, besides reducing infarct size and ventricular arrhythmias, IPC was also shown to improve left ventricular ejection fraction and survival of the myocardium. These effects were meditated by a vast number of IPC associated molecules, including ROS and NO (reviewed in [146]). Additional studies have demonstrated the protective effects of IPC in various organs such as the kidney, brain, liver, and intestine, summing up to over 7700 studies on IPC in PubMed.

In the first study investigating post-conditioning, the left anterior descending artery (LAD) in dogs was reversibly occluded for 60 min, reperfusion was initiated for 30 s followed by 30 s reocclusion, repeated for three cycles (3 min total intervention) (Fig. 5C). These intermittent intervals of I/R are strikingly similar in duration to three consecutive apneic events. Postconditioning was as effective as preconditioning in reducing infarct size and preserving endothelial function [147].

In some instances protection by pre- or post-conditioning might be activated in cardio- and cerebro-vascular conditions in OSA, as well as in other organs. Accordingly, it should be noted that despite the high prevalence of cardio–cerebral morbidities and the detrimental mechanisms associated with OSA there are some epidemiological studies that suggest the possible activation of protective mechanisms. Thus, even though the demonstrated association of SDB and cardiovascular morbidities would suggest worse outcomes in OSA patients, particularly in the setting of AMI, this was not

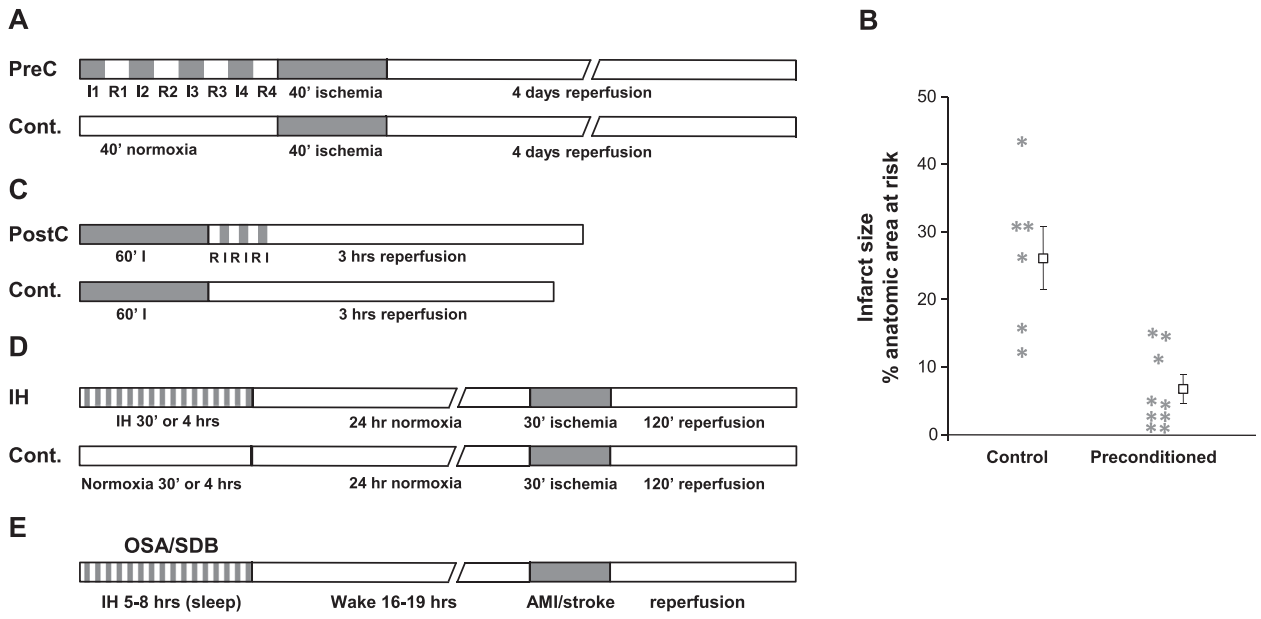

Fig. 5. Ischemic conditioning. (**A**) The experimental design used by Murry et al. [145] Preconditioned animals underwent four 5 min circumflex coronary occlusions, each separated by 5 min of reperfusion, followed by a sustained 40 min occlusion (global ischemia) and four days reperfusion recovery. Control animals underwent a single 40 min occlusion. (**B**) infarct size in preconditioned and control animals according to the experimental design in (A). (**C**) Postconditioning protocol according to Zhao et al. [147] (**D**) The experimental design for acute IH treated rats by Beguin et al. [173] (**E**) Adaptation of the experimental design in (D) for AMI or stroke in patients with OSA.
Cont, controls; IH, intermittent hypoxia; I, ischemic occlusion; OSA, obstructive sleep apnea; PreC, preconditioned; postC, post conditioning; R, reperfusion; SDB, sleep disordered breathing.

shown in all studies [6,136,148–150]. Furthermore, while several studies reported an increased all-cause mortality in OSA patients, there was no association with ischemic heart disease [151]. Marshall et al. [152] did not find an association between death and cardiovascular morbidity, and there are paradoxical findings on significantly improved post-operative survival of OSA patients [153,154], as well as on increased survival of elderly with mild OSA [155].

Molecular mechanisms of I/R: effects on the heart

Consequences of ischemia to the heart. During the ischemic period, the lack in oxygen and nutrient supply alter the cellular metabolism to anaerobic glycolysis. Subsequently, various complex metabolic and molecular changes occur, primarily in the mitochondria, which are the main source of ROS in cardiomyocytes. For instance, inhibition of oxidative phosphorylation, induction of mitochondrial membrane depolariziation, depleted ATP levels, increased ROS, Ca^{+2} overload and increased lactate production. The latter lowers the intracellular pH and therefore inhibits the contractile function of the myocardium [143].

Consequences of reperfusion to the heart. During the reperfusion–reoxygenation period the mitochondrial electron transport chain is reactivated, and generates ROS, which induce cell and tissue injury by the notoriously known I/R injury associated ROS. Also, lactic acid levels, the physiological pH and the mitochondrial membrane potential are rapidly restored. The increased generation of ROS/RNS promotes mitochondrial permeability transition pore (mPTP) opening. Apparently, myocardial injury during reperfusion is mediated by many critical factors including oxidative stress, intracellular Ca^{+2} overload, the rapid restoration of the intracellular pH, and inflammation. A burst of ROS is generated during the first few minutes of the reperfusion through the mitochondria. It is likely that increased ROS are also produced by other sources mentioned previously including xanthine oxidase and Nox from activated endothelial cells and leukocytes. However, prolonged mPTP opening is considered as one of the endpoints of myocardial damage causing loss of cardiomyocyte function and viability. Thus, both ROS an mPTP are considered as fundamental mechanisms affecting mitochondria and myocardial infarction [143]. Oxidative burst mediates myocardial reperfusion injury by damaging all cellular components directly or indirectly through mPTP opening. Timely myocardial reperfusion is essential to rescue the myocardium and restore contractile function. However, if the acute myocardial ischemic period is prolonged for more than 20 min, massive cardiomyocyte death occurs [138,156]. Along with the above mentioned alterations occurring due to I/R, additional consequences result from altered gene expression and signal transduction pathways affected by ROS, which may induce tissue injury or protection.

Molecular mechanisms promoting IPC

Although protective IPC mechanisms are complex and not entirely understood, and involve multiple molecular and cellular mechanisms interacting to reduce cellular injury, a great number of molecules and signaling pathways were identified. These include triggering substances that are generated during IPC. For instance, adenosine, the breakdown product of ATP, which activates purinogenic signaling. Extracellular conversion of ATP to adenosine has a central role in attenuating sterile inflammation and protecting vascular endothelium and cardiac myocytes during I/R injury [157]. The adenosine mediated cardioprotective response involves stabilization of HIF-1α and induction of its downstream glycolytic genes to generate ATP during I/R [158]. Additional molecules participating in cardioprotection include bradykinin, opioids, cytokines, NO as well as ROS which trigger molecules in a bimodal way. Prior to the ischemia, ROS act as signaling molecules mediating cardioprotection, while during the ischemic period ROS impact on cell death. As a result of the above mentioned substances, various mediators and kinases are activated. These include protein kinase C (PKC), phosphatidylinositol-3-kinase (PI3K)/Akt kinase and Erk1/2,

which in turn activate critical transcription factors such as NFκB, AP-1, Nrf2 and stabilize HIF-1α [146]. Besides generating ATP by upregulating glycolytic enzymes, HIF-1α is required for further preconditioning effects. Its stabilization also allows for upregulation of downstream genes as VEGF, erythropoietin (EPO), iNOS and heat shock proteins (HSPs). Upregulation of these genes supports angiogenic mechanisms promoting IPC such as endothelial progenitor cell activation and heart collateralization [159–161].

The NFκB is a pleiotropic transcription factor affecting many cellular processes and is mostly known for its deleterious effects by upregulating inflammatory/immune cascades. However, it was also shown to participate in cardioprotection by remote preconditioning associated with I/R [162]. Yet, the role of NFκB in cardioprotection is complex and remains poorly understood. Conversely, Nrf2 which regulates the expression of constitutive and inducible anti-oxidant enzymes is a much more established participant in cardioprotection against I/R injury. In a number of recent studies it was shown to protect hearts by IPC through the induction of antioxidant enzymes of the glutathione pathway and by modulating phase II detoxifying enzymes through p38 MAPK and PI3K/Akt activation [163,164]. Moreover, rabbit hearts were protected from I/R injury by activating IPC mechanisms through PKC activation which induced Nrf2 and antioxidant defenses. Consequently, this resulted in a better recovery of mechanical function, and increases in SOD, catalase, and GSH/GSSG ratio (more of the reduced form). Also, oxidative stress measured by MDA was lowered compared to non-IPC treated rabbit hearts. In parallel, the antioxidant enzymes HO-1 and mitochondrial SOD were also increased [165]. In addition, in transgenic hearts of mice overexpressing extracellular SOD (SOD3), I/R injury was attenuated by reducing infarct size and improved functional recovery. Accordingly ROS levels were decreased and NO bioavailability was increased [166]. These findings emphasize the importance of the extracellular SOD isoform in protecting from I/R injury. Jointly, these studies clearly indicate the importance of endogenous antioxidant defenses in protecting against I/R injury and cardiovascular morbidities. Additional molecules and the pathways they trigger in IPC are reviewed in [146]. Thus, it should be noted that the levels of a great number of the molecules participating in IPC mentioned above are also increased/activated in OSA/SDB and/or in animal models mimicking OSA. Just to name a few; adenosine, bradykinin, p38 MAPK, NFκB, HIF-1α, HSPs, and Nrf2.

Cellular angiogenic mechanisms promoting IPC

Besides regulating multiple molecular pathways, ROS are also crucial regulators in angiogenesis, a process characterized by the formation of new blood vessels from preexisting vessels. They do so by activating endothelial progenitor cells (EPCs) which consequently promote coronary collateral formation [167–169]. Both molecular and cellular mechanisms regulating IPC are intertwined and activate each other through upregulation of HIF-1α and its downstream genes VEGF and EPO. As such, EPCs replace dysfunctional endothelium and enhance tissue repair after ischemic vascular injury. Of particular importance is their incorporation into active sites of angiogenesis or at sites of myocardial infarction into foci of neovascularization [159–161]. Thus, angiogenesis and coronary collateral formation contribute to enhance cardio-protection. In light of these findings the importance of hypoxia based strategies to induce angiogenesis for tissue regeneration is beginning to unfold as a new therapy for ischemia associated morbidities such as AMI, stroke, and peripheral vascular disease [170]. Since HIF-1 mediates adaptive and pathological processes in the heart as well as signaling, targeting HIF-1 is being considered as a potential therapeutic modality in cardioprotection [171].

Protective mechanisms in animal models of IH and OSA/SDB

Activation of molecular protective mechanisms in animal models of IH

Most of the studies demonstrating IH-associated beneficial effects in the heart and brain are derived from animal models mimicking OSA. In a study by Naghshin et al. [172], cardiac function was characterized in C57Bl/6J mice subjected to chronic IH during four weeks (at 5% O_2, for 30 s/min, 60 events/h, for 8 h a day). Surprisingly, left ventricular contractility was increased. In a series of studies conducted by Beguin, Belaidi and colleagues [173–175], rats were subjected to various paradigms of acute IH (see Fig. 5D). Compared to normoxia, the IH applied improved the tolerance of the myocardium to ischemia and induced delayed preconditioning by reducing infarct size in isolated rat hearts. The decreased infarct size was dependent on the depth (10% O_2) and the duration (4 h) of the IH. Applying a more severe IH (5% O_2), or treatment with continuous hypoxia for the same duration did not improve infarct size [173]. Thus, IH exerted **dichotomous** effects on the heart depending on its severity. In follow-up studies, using the same effective IH protocol, besides the reduced infarct size, myocardial HIF-1α and iNOS gene expression were upregulated and coronary flow was improved. Moreover, by inhibiting iNOS, the cardioprotective effect of the IH was lost [174]. This delayed preconditioning effect induced by IH was mediated by PKC and triggered by p38 MAPK and Erk1/2 [175]. A more recent study conducted on rats exposed to IH for four weeks demonstrated also tissue specific **dichotomous** effects. While IH increased serum levels of oxidative stress and inflammatory markers, cardiac oxidative stress and inflammatory markers were attenuated, and protective molecules such as Nrf2 and HO-1 were increased. Yet cardiac pro-apoptotic Bax levels were also increased [126]. These latter findings indicate that in response to IH, protective mechanisms as well as apoptotic processes were activated in the heart. In a more recent study investigating the role of chronic IH on ischemic stroke, Jackman et al. [70] also demonstrated an IH dependent **dichotomous** effect on the brain which was dependent on the severity of the chronic IH (treated for $\leq$35 d, 8 h a day, 20 hypoxic episodes/h, each lasting 90 s – at 10% or 6% O_2) and was associated with mitochondrial ROS. While chronic IH using 10% O_2 was neuroprotective and significantly reduced infarct volume compared to controls, IH using 6% O_2 exacerbated tissue damage. Accordingly, compared to controls, mitochondrial ROS was decreased by treatment with IH at 10% O_2 and was increased by treating mice with IH at 6% O_2. Additionally, IH at 10% O_2, attenuated inflammatory adhesion molecules such as ICAM-1, VCAM-1 and iNOS, whereas at 6% O_2 these inflammatory markers were increased. These findings comply with Kaczmarek et al. [111] data, described earlier, on the differential mRNA expression of VCAM-1, eNOS, HIF-1α and VEGF in severe compared to mild OSA patients. Of note, treatment with IH after transient focal ischemia in rats (resembling post-conditioning protocols), induced hippocampal neurogenesis, and c-Fos expression via HIF-1 and MAPK signaling [176]. Also, in patients with OSA a bilateral enlargement of hippocampi was found compared to matched controls. It was suggested that hippocampal hypertrophy in OSA patients might represent a compensatory response to the detrimental effects of the IH on the brain [177]. This finding is in line with preclinical studies providing evidence on hippocampal neurogenesis in response to IH, which resulted in increased volume and thickness [178–180]. Jointly, these findings demonstrate some of the beneficial effects of IH on the heart as well as specific brain areas.

Additional animal studies further support the potential beneficial effects of IH also to other organs. Pretreatment with IH improved respiratory [181] and neurorespiratory motor function as well as neuroplasticity after spinal injury [182]. These effects were mediated

through ROS, HIF-1α and VEGF, as also described earlier for the cardio- and cerebro-vascular systems. Based on these studies the authors suggested harnessing IH as a treatment modality for spinal injury and amyotrophic lateral sclerosis (ALS). This line of evidence is beyond the scope of this manuscript, and is described elsewhere [181–184].

From the animal studies described herein it is suggested that some IH patterns mimicking OSA might be beneficial to the heart and brain while others are detrimental. Repeatedly, the depth, durations and intermittence of the hypoxia are of utmost importance and thus, have to be delineated. Altogether, the mechanisms described clearly attest to the involvement of ROS, HIF-1α, iNOS, various kinases, transcription factors and redox pathways in IH modeling OSA [173–175,185,186] and concur with the classical IPC phenomenon and the mechanisms it activates, as described earlier. These animal studies utilizing IH shed a light on the potential molecular mechanisms involved in IH associated cardio- and neuro-protection. In some of these models, the IH can be viewed as serving a non-lethal preconditioning effect before the occurrence of a lethal global ischemia as in AMI or stroke (as depicted in Fig. 5A and E) [173–175].

Activation of cellular cardio-protective mechanisms in OSA/SDB

Thus far, molecular based studies investigating potential cardioprotective effects of IH in patients with OSA/SDB were not reported, likely due to technical and ethical constraints. However, many of the molecules participating in IPC are also upregulated in OSA/SDB. In addition, the animal studies described above support the feasibility of this mechanism in cardio- and neuro-protection. Moreover, more recent epidemiological studies and cellular based studies point to the importance of exploring this possibility in OSA.

It is widely recognized that the IH associated with OSA has major unfavorable effects, mainly in severe patients. Hence, not all OSA patients develop cardio–cerebro-vascular consequences as indicated by morbidities and mortality endpoint studies. Thus, the question arises – why not all patients with OSA develop co-morbidities or die. It should be realized that various protective mechanisms might be activated as well, in order to counteract and protect the organism.

From the few studies reported thus far it is indicated that in some instances OSA/SDB may confer cardio-protection. Hence, two main lines of investigation were reported on cellular mechanisms promoting IPC: 1) the involvement of endothelial progenitor cells (EPCs) through increase in their activity and function and 2) increased coronary collateral formation. Both were demonstrated in cardiovascular populations with OSA/SDB. Apparently, some of the molecular cardioprotective mechanisms described in animal models subjected to IH, namely ROS, HIF-1α stabilization and upregulation of its downstream genes (VEGF, EPO and iNOS) can stimulate increases in EPCs numbers and functions resulting in coronary collateral formation [159–161,167–169].

Since EPCs are mobilized by tissue ischemia or hypoxia, as well as by VEGF and HIF-1α dependent pathways associated with hypoxic preconditioning [187], it is reasonable to assume that in response to IH/OSA/SDB their numbers and/or functions might be altered. To date, however, the findings describing EPCs in OSA/SDB and in animal models of IH are still limited and discordant. For instance, EPCs (CD34+) numbers were shown to positively correlate with ODI and the duration of oxygen desaturation [188], however, in another study their repair capacity was reduced [57], and in children with OSA, circulating EPCs numbers were dependent on the magnitude of the endothelial dysfunction [189]. It is likely that the severity of OSA, the magnitude of the oxidative stress, as well as the definition of EPCs are important factors. (See also reviews in [190,191]).

In a recent study from our laboratory, EPCs numbers and angiogenic functions were determined in matched groups of AMI patients with and without SDB [192]. The study was specifically designed to test whether the nightly alternating hypoxic/normoxic events in OSA/SDB may act as non-lethal sequential episodes of I/R in the form of pre-conditioning [193], thus, protecting OSA/SDB patients mainly in case of having a major acute lethal I/R episode – like AMI or stroke. Patients hospitalized after an AMI were subdivided according to the severity of SDB into two groups; AMI patients without SDB (AMI-only, ODI $\leq$ 5 events/h) and AMI patients with SDB (AMI-SDB ODI > 5 events/h). EPCs (CD34+VEGF-R2+) numbers and proliferative and angiogenic properties as well as VEGF levels in monocytes were increased in AMI patients with SDB compared to AMI-only patients. However, we should cautiously note that the AMI-SDB patients in this study were only of mild-moderate severity. Thus, an entirely different picture might be obtained with severe SDB patients if considering the opposite effects of low vs. high ROS levels. These increases in EPCs numbers, their increased proliferative and angiogenic properties and the higher VEGF levels noted in monocytes may explain the higher collateralization found in coronary artery disease (CAD) patients with OSA as reported by Steiner et al. [194]. These two studies indicate that in cardiovascular patient populations, OSA may exert a preconditioning effect [195]. Another line of evidence which supports a higher angiogenic activity in patients with OSA stems from a study by Wahlin-Larsson et al. [196], which demonstrated an increase in skeletal muscle microvascularization (capillary network) of patients with OSA that was also correlated with increased VEGF-A expression.

It is indicated from various studies that EPCs numbers and functions are strongly associated with increases in coronary collateral vessels. Accordingly, in patients with poor collateralization EPCs numbers and angiogenic properties were shown to be low, whereas in patients with good collateralization EPCs numbers and angiogenic properties were higher [197,198]. Since angiogenesis can be considered "good" or "bad" depending on the individual pathological setting, the higher angiogenic activity observed in OSA may confer protection to the heart by increasing blood supply in cardiovascular cases. On the other hand, increased vascularization may also promote the development of more aggressive cancers in OSA [16].

Potential pitfalls in identifying IPC in OSA/SDB

A great number of factors can hamper IPC processes in OSA/SDB. First, the presence of comorbidities and confounders. These are known to attenuate the effects of IPC. Most or all of these confounders and comorbidities are present in patients with OSA and in those needing cardioprotection therapy [199,200]. Aging was specifically shown to abolish the protective effects of IPC by preventing the restoration of endothelial function [201]. Second, inter-individual differences in the response to an identical hypoxic stimulus due to variations in oxygen regulated gene expression. This implies that the same hypoxic stimulus, when applied to cells or tissues from different individuals, can result in diverse responses in hypoxia sensitive transcription factors, downstream gene products, and transduction pathways. This was shown for VEGF and other downstream genes of HIF-1α, suggesting that the source of this variation resides within the HIF system itself [202]. Such inter-individual differences in the hypoxic response between subjects by the induction of VEGF are also exemplified in an earlier study reporting on a positive and highly significant correlation between the VEGF response to hypoxia in monocytes harvested from CAD patients, and the presence of collaterals in the heart [203]. These inter-individual differences are further exemplified by demonstrating that HIF polymorphism was associated with the development of collaterals in patients with ischemic heart disease [204]. Thus, it is suggested that variations in the HIF-1α genotype may affect the development of coronary artery collaterals in patients with significant coronary

artery disease. Jointly, these heterogenic responses to identical hypoxic stimuli due to genetic variations may explain the variable angiogenic responses between individuals, and might be manifested mainly in disease states. There are also epigenetic differences between individuals as well as nutritional, environmental and life style related variables. Thus, clarifying the mechanisms of preconditioning and the factors that control them in OSA/SDB/IH may help in harnessing this potentially important therapy as a treatment modality. Third, identifying the effective patterns of IH, or, the "protocol" which may induce IPC. Fourth, the effect of IPC in SDB/OSA might be mainly manifested in the context of an additional severe and prolonged ischemic event such as AMI, stroke, or trauma (as depicted in Fig. 5E). Thus, it is likely that in order to identify IPC in OSA, the experimental strategies should be modified.

Conclusions

Reactive oxygen and nitrogen species (ROS/RNS) have come to occupy an incredibly central role in physiological and pathophysiological conditions. These depend on the type of ROS/RNS produced, the intracellular site of production, the microenvironmental antioxidant activity and their concentrations. At low concentrations, they can act as "good" by regulating vital cellular functions, at higher concentrations they act in a "bad/ugly" manner, by promoting oxidative stress, cellular injury and a great number of diseases. The specific stimulus initiating ROS is of utmost importance as well. While leukocytes produce large quantities of ROS/RNS in infectious and inflammatory conditions, which are beneficial and protective to the host, sterile inflammation or I/R activating leukocytes to produce larger amounts of ROS, can be damaging to surrounding tissues. Similarly, I/R inflict injury, but also activate protective preconditioning effects. Yet, to date, unlike in animal models mimicking OSA the mechanisms of preconditioning in patients with OSA remain unclear. Likely they involve the ability of cells to maintain an appropriate redox balance via upregulation of distinct sets of cytoprotective genes responsible for adapting and mitigating oxidative stress.

It should be emphasized that along with the wide range of hazardous effects of OSA/SDB, particularly in severe and younger patients, some potentially protective mechanisms are activated. This is particularly evident in AMI patients with co-existent OSA/SDB. It is in the nature of biological systems to compensate and counteract hazards in an attempt to restore the well-being of the organism. The severity of OSA is likely to play a major role in this cardioprotection. No less important is the genetic makeup. Having the "right" polymorphic genes associated with "proper" ROS/RNS production, and induction of the "proper" protective antioxidant pathways may result in a "proper" redox state – which will define the action of ROS/RNS as "good" rather than "bad" or "ugly".

Although much has been done in the last decade regarding oxidative stress in OSA/SDB and its implications to associated conditions and co-morbidities, much more has yet to be done in order to fully understand the impact of oxidative stress and ROS/RNS in this syndrome. Animal models and healthy humans exposed to IH in vivo, OSA/SDB patients with concomitant AMI, as well as in vitro studies, can be instrumental in elucidating these processes. The complex interactions between various ROS/RNS/antioxidant mechanisms and various redox sensitive transcription factors and signaling pathways affected by IH should be further investigated. Tissue specificity is immensely important to fully apprehend the activity and functions of ROS/RNS. Thus, delineating differential tissue/organ specific effects of IH on the redox balance may help to explain apparently contradictory findings. Inter-individual differences in ROS/RNS levels resulting from specific oxidative metabolism gene variants, dietary consumption and other lifestyle related variables such as physical activity should be considered as well. Based on findings indicating the presence of ischemic preconditioning in IH/OSA, the potential contribution of IH to the development of such protective mechanisms should be also realized, in order to be able to harness it for therapeutic purposes. Accordingly, identifying effective patterns of IH combined with personalized medicine to determine the individual redox state may help to identify patients at risk.

Practice points

1) Obstructive sleep apnea (OSA) is associated with oxidative stress which likely contributes to various cardiovascular risk factors and morbidities.
2) In some instances OSA/IH may confer cardio- and neuroprotection by activating mechanisms of ischemic preconditioning. This is particularly evident in patients with cardiovascular morbidity and concomitant OSA, and in mild-moderate OSA.
3) In animal models mimicking OSA a dichotomous effect is noted. Exposure to mild-moderate IH corroborates activation of cardio- and neuro-protection, whereas exposure to severe IH induces damage to these organs.
4) Preconditioning is complex and involves a multitude of molecules, transcription factors, and transduction pathways, and thus is not entirely elucidated. However, it likely involves the cells' ability to maintain homeostatic redox balance by adapting to oxidative stress via upregulation of distinct sets of cytoprotective genes responsible for promoting the cells' antioxidant capacity.
5) Paradoxical data from epidemiological mortality-endpoint studies may support the existence of cardioprotective mechanisms in OSA.
6) New experimental strategies should be implemented in order to identify protective mechanisms in OSA.

Research agenda

Along with the wide range of hazardous effects inflicted by oxidative stress in OSA, particularly in severe and younger patients, in some instances some potentially protective mechanisms are activated. This is particularly evident in AMI, cardiovascular and stroke patients with co-existent OSA, and is also corroborated by animal studies.

1) Identify tissue/organ-specific sources of ROS/RNS/antioxidant mechanisms in OSA to differentiate between "the good, the bad and the ugly".
2) To specifically target mitochondrial function under intermittent hypoxia (IH).
3) Identify genetic variants in ROS/RNS producing enzymes (Nox, NOS, xanthine oxidase) and HIF-1α affecting oxidative stress in OSA.
4) Clarify the role of Nrf2 in cardio-/neuro-protection in OSA.
5) Identify patterns of IH that exert beneficial-protective effects on the heart and brain and determine how various OSA patterns of IH (mild-moderate vs. severe) can affect mechanisms of ischemic preconditioning or damage.

6) Determine which are the specific parameters of IH that confer cardiovascular risk.
7) Implement studies to determine the efficacy of antioxidant treatment in OSA and identify who might benefit from such a treatment.
8) Develop tests to determine oxidant/antioxidant gene array for identifying the individual redox state of OSA patients to be used for personalized medicine, to determine who is at risk.
9) Identifying markers of IPC in patients with OSA should concentrate on those after an ischemic event such as AMI or stroke.

Acknowledgments

This paper is dedicated to Lloyd E. Rigler (1915–2003), who passionately supported sleep medicine research. The support of the Lloyd E. Rigler – Lawrence E. Deutsch Foundation to L. Lavie and P. Lavie is gratefully acknowledged. The assistance of Prof. P. Lavie in reviewing the manuscript and his helpful suggestions are much appreciated. The help of Dr A. Polyakov in preparing the Figures is gratefully acknowledged. I would also like to thank the staff of the Lloyd E. Rigler Sleep Apnea Research Laboratory for their continuous support.

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